NOTES AND LITERATURE

WORK ON GENETIC PROBLEMS IN PROTOZOA AT YALE

Two ideals are commonly represented in the practise of university laboratories. Some concentrate upon a unified set of problems, endeavoring thus to make a definite mark upon science; others cultivate breadth, the different workers taking up problems lying in diverse fields. The work done in recent years at the Yale Zoological Laboratory by Professor L. L. Woodruff and his associates is an interesting example of the former type; the present is an attempt to give a unified survey of this work, which has been directed with concentration and effectiveness upon the general questions of reproduction in unicellular animals.

The work on these matters may be represented as a tree with a single trunk and diverging branches. The trunk consists of the study of Woodruff's culture of a single line of *Paramecium*, begun in 1907, to test the hypothesis that death is a necessary consequence of continued reproduction without conjugation. This study was itself an outgrowth of an investigation (the seed of the tree) made by Woodruff as a student under the direction of the investigator who has been chiefly responsible for the recent revival of work on the more general problems of reproduction in the Protozoa, Professor Calkins, of Columbia University. This first investigation (1) led to results in agreement with the views of Maupas and of Calkins, that continued reproduction without conjugation results inevitably in death.

> All the cultures give incontestable proof that the species studied [*Oxytricha fallax, Pleurotricha lanceolata,* and *Gastrostyla steinii*] pass through cyclical periods of general vitality. The periods of depression lead to death if the culture is subjected to the same environment. Minor fluctuations also occur which may be called "rhythms."
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> A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous.
>
> A cycle is a periodic rise and fall of the fission rate, extending over a varying number of rhythms, and ending in the extinction of the race unless it is "rejuvenated" by conjugation or changed environment (1, page 627).

Woodruff, however, felt that the matter needed further test, particularly with relation to the part played by environmental

conditions, as compared with that dependent upon internal factors. Suspecting that the ultimate death might be due rather to the constancy of the conditions than to anything inherent in the process of living, he set in progress on May 1, 1907, a line derived from a single individual of *Paramecium aurelia,* keeping it under varied conditions. That is, the culture medium was altered from day to day. This line was found to reproduce actively, without degeneration and without conjugation. From time to time Woodruff has published brief papers showing the progress of this line and the relation of the facts to general problems. Such bulletins have been issued at the 465th generation (5); then at generations 490 (3), 1,185 (6), 1,238 (7), 1,795 (9), 2,121 (10), and 3,029 (22). The culture at last accounts had been in progress five years, during which time the animals had reproduced 3,029 times without conjugation; the potential number of progeny produced being represented by 2 raised to the 3,029th power (a number composed of 912 integers), and constituting a volume of protoplasm equal to 10^{1000} times the volume of the earth (22, page 123). Woodruff well concludes:

> I believe this result proves beyond question that the protoplasm of a single cell may be self-sufficient to reproduce itself indefinitely, under favorable environmental conditions, without recourse to conjugation, and clearly indicates that senescence and the need of fertilization are not primary attributes of living matter (22, page 123).

This conclusion has been supported by the work of other investigators (notably by that of Enriques), but all will agree that the mainstay of this most important generalization is this work of Woodruff.

Under varied conditions the reproductive power of this line thus showed itself to be indefinitely great. Now arose the question whether the variation of the conditions was the essential point, or whether the death in a constant hay infusion may not be due to a lack in the hay of elements essential to the prolonged life of the cultures; in other words, whether it may not be a case of slow starvation. To test this, Woodruff and Baitsell (15) on October 1, 1910, separated from the line living under varied conditions a set which was kept in a constant medium of ¼ per cent. beef extract. After seven months the authors report that this was "practically as favorable a medium for the reproduction of this pedigree culture of *Paramecium aurelia* as the 'varied' environment, and therefore . . . it appears fair to conclude that

it is the 'composition' of the medium rather than the changes in the medium which is conducive to the unlimited development of this culture without conjugation or artificial stimulation'' (15, page 141).

From this basic investigation, giving conclusive results on an ancient and fundamental problem, have grown branch studies by Woodruff and his associates on a large number of diverse factors affecting reproduction. This work has been done mainly on *Paramecium,* as a contribution to the general effort to get the genetic physiology of one type animal fully cleared up, but other infusoria have likewise been dealt with. We may divide these studies into (1) those on internal factors and (2) those on external factors.

1. *Internal Factors.*—In his first paper (1) as we saw, Woodruff distinguished certain small changes in the reproductive rate, which he called rhythms. The question comes up as to whether these, like the changes resulting in death, may not be due to something in the environmental condition, perhaps to fluctuations in these conditions. This problem was attacked by Woodruff and Baitsell (16). Their result was that practically constant conditions of the environment tend to bring out the rhythms more clearly, from which it is concluded that they are due to causes within the organism. A possible chance for doubt of this conclusion arises from the question whether the precautions taken to keep the bacterial content of the cultures uniform were adequate; certain work done in the Zoological Laboratory of the Johns Hopkins University indicates that they were not—in which case the fluctuations in the reproductive rate might be due to variations in the bacterial content of the medium.

Baitsell's study (13, 23) of the effects of conjugation between closely related individuals in *Stylonychia pustulata* belongs here. It was found that after such conjugations the animals do not continue to reproduce. Baitsell summarizes as follows:

> The experiments show conclusively, it is believed, that conjugation is induced by *external* conditions affecting the organisms, and bears no relation, in this form at least, to a particular period of the life cycle.
>
> It is suggested that infertility of syzygies in these cultures is the result of the fact that the gametes had an identical environmental history (23, page 74).

With regard to the second suggested conclusion, doubt may be raised, since it was not shown that under the conditions gametes

with diverse environmental history give more fertile pairs; possibly conjugation involves regularly the death of a large proportion of the gametes.

Here perhaps belongs also the study by Woodruff (14) showing that *Paramecium caudatum* and *Paramecium aurelia* are distinct species:

> Since one of the crucial tests of a species is its ability to breed true to type indefinitely, *aurelia* and *caudatum* have adequately met this test during more generations than any other animal under observation (14, page 237).

2. *External Factors.*—Studies of the effects of a long list of external factors on reproduction have branched out from the main trunk given by the study of the life cycle. In his "seed paper" of 1905 (1), Woodruff had included a number of experiments with various chemical and physical agents, showing particularly that Protozoa are extremely sensitive to solutions of electrolytes. He followed this up in 1908 (4) with a study of the effects of alcohol. This showed that:

> (1) Minute doses of alcohol will decrease the rate of division at one period of the life cycle and increase it at another period of the life cycle. (2) When alcohol increases the division rate the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control. . . . (4) Treatment with alcohol lowers the resistance of the organisms to copper sulphate (4, page 104).

Woodruff and Bunzel (8) further undertook a precise study of the directly destructive effects of various salts and acids on Paramecia taken from the pedigree culture serving for the trunk experiments. The results of this work, not bearing directly on reproduction, lie a little to one side of the main stream of experimentation; the conclusion is:

> Considered as a whole, the results of the experiments indicate a marked parallelism between the order of toxicity of the various cations toward *Paramecium* and the ionic potential of the ions employed (8, page 194).

A considerable number of studies (11, 12, 17, 18, 19, 20, 21) are devoted to analysis of the effects of the environmental conditions to which the animals are subjected in their natural lives, —the culture media—upon reproduction and vitality. A paper by Woodruff (12) on "The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction" concludes:

(1) The rate of reproduction of *P. aurelia* and *P. caudatum* is influenced by the volume of the culture medium, within the limits tested [*i. e.*, 2, 5, 20 and 40 drops of varied environment medium] and the greater the volume the more rapid is the rate of division. (2) *Paramecia* excrete substances which are toxic to themselves when present in their environment, and these substances are more effective when the organisms are confined in limited volumes of the culture fluid. (3) The excretion products of *Paramecium* play an appreciable part in determining the period of maximum numbers, rate of decline, etc., of this animal in hay infusions (12, page 581).

A careful study of the effects of changes of temperature on reproduction made by Woodruff and Baitsell (17) showed that the temperature coefficient (factor by which the rate of reproduction is multiplied when the temperature is raised ten degrees) is approximately 2.7, so that the rate of cell division is influenced by the temperature in a manner similar to that for a chemical reaction.

These studies of environmental action had shown Woodruff that different races of *Paramecium* are adapted to different conditions, and that this throws light on the diverse results reported by different observers. In a paper of 1911 (18), he concludes:

(1) The discrepant results of various workers on the longevity of *Paramecium* are in all probability due to variation in the cultural demands of the race isolated for study. (2) It is probable that most, if not all, normal individuals have under suitable environmental conditions, unlimited power of reproduction without conjugation or artificial stimulation (18, page 65).

In this second statement much is involved in the word "normal"; the experience of the Johns Hopkins Laboratory is that some lines die out after a time, even though they may at first multiply in the usual way.

The study of cultural action was next made general and extended to the other organisms in the infusorian cultures, by a careful examination of the source and sequence of development of the organisms usually found in the cultures of decaying vegetation. The conclusions are of practical interest for the laboratory worker. Woodruff finds that Protozoa are rarely introduced from the air; and that *Paramecium* is not introduced dry, on hay or otherwise.

Air, water, and hay are all sources from which Protozoa are derived, and increase in importance in the order given. Of these three, however,

air is practically a negligible factor in seeding infusions (19, page 263).

The order in which different common forms most frequently appear, reach their maximum, and disappear in hay infusions is shown in the following list (taken from 19, page 243).

Appearance	Maximum	Disappearance
1. *Monads.*	1. *Monads.*	1. *Monads.*
2. *Colpoda.*	2. *Colpoda.*	2. *Colpoda.*
3. *Hypotrichida.*	3. *Hypotrichida.*	3. *Hypotrichida.*
4. *Paramecium.*	4. *Paramecium.*	4. *Amœba.*
5. *Vorticella.*	5. *Amœba.*	5. *Paramecium.*
6. *Amœba.*	6. *Vorticella.*	6. *Vorticella.*

As hay infusion is the typical culture medium for such organisms, a study was made by Fine (20) of its chemical properties, with particular relation to the acidity, the purpose being to correlate, so far as possible, the chemical conditions with the protozoan sequence. The paper concludes:

> The acidity of hay infusions is essentially due to bacteria, their efficiency in producing acid being governed by the concentration of the infusion in acid-yielding materials. The protozoa play a relatively small part in the production of acid.
>
> The sequence of protozoa and the course of the titratable acidity possess no intimately mutual relation. Either may vary within wide limits without appreciably influencing the course of the other.

This line of work is evidently still under active treatment, since we note that the *Journal of Experimental Zoology* promises a paper by Woodruff on "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions."

The problems of reproduction, age and death are bound up in recent theories with those of the size of cells and nuclei; a paper on this aspect of matter in the same journal is likewise promised from Woodruff.

Among the laboratories of this country which have made a definite mark on some unified problem of general interest (and such are happily now becoming numerous), certainly a most honorable place must be accorded to this work done at Yale by Woodruff and his associates.

A. R. Middleton

Zoological Laboratory of the Johns Hopkins University

CHRONOLOGICAL LIST OF PAPERS

(In the above review the papers are referred to by the serial numbers here given in parentheses. The papers in the *Proceedings of the Society of Experimental Biology and Medicine* and in *Science* are merely short abstracts on work published in full in other papers.)

(1) *Woodruff, L. L.,* An Experimental Study on the Life History of Hypotrichous Infusoria, *Journ. Exp. Zool.,* 2: 585–632, November, 1905.—(2) *Id.,* Variation During the Life Cycle of Infusoria and its Bearings on the Determination of Species, *Science,* 25: 734–735, May 10, 1907.—(3) *Id.,* The Life Cycle of *Paramecium, Proc. Soc. Exp. Biol. and Med.,* 5: 124, May 20, 1908.—(4) *Id.,* The Effect of Alcohol on the Life Cycle of Infusoria, *Biol. Bul.,* 15: 85–104, 1908.—(5) *Id.,* The Life Cycle of *Paramecium* when Subjected to a Varied Environment, AMER. NAT., 42: 520–526, August, 1908.—(6) *Id.,* Studies on the Life Cycle of *Paramecium, Proc. Soc. Exp. Biol. and Med.,* 6: 117–118, May 26, 1909.—(7) *Id.,* Further Studies on the Life Cycle of *Paramecium, Biol. Bul.,* 17: 287–308, September, 1909.—(8) *Woodruff, L. L., and Bunzel, H. H.,* The Relative Toxicity of Various Salts and Acids toward *Paramecium, Amer. Journ. Physiol.,* 25: 190–194, December, 1909.—(9) *Woodruff, L. L.,* On the Power of Reproduction without Conjugation in *Paramecium, Proc. Soc. Exp. Biol. and Med.,* 7: 144, May 18, 1910.—(10) *Id.,* Two Thousand Generations of *Paramecium, Archiv f. Protistenkunde,* 21: 263–266, 1911.—(11) *Id.,* The Effect of Culture Medium Contaminated with Excretion Products of *Paramecium* on its Rate of Reproduction, *Proc. Soc. Exp. Biol. and Med.,* 8: 100, April 19, 1911.—(12) *Id.,* The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction, *Journ. Exp. Zool.,* 10: 557–581, May, 1911.—(13) *Baitsell, G. A.,* Conjugation of Closely Related Individuals of *Stylonychia, Proc. Soc. Exp. Biol. and Med.,* 8: 5, May 17, 1911.—(14) *Woodruff, L. L., Paramecium aurelia* and *Paramecium caudatum, Journ. Morphol.,* 22: 223–237, June, 1911.—(15) *Woodruff, L. L., and Baitsell, G. A.,* The Reproduction of *Paramecium aurelia* in a "Constant" Culture Medium of Beef Extract, *Journ. Exp. Zool.,* 11: 135–142, July 5, 1911.—(16) *Id.,* Rhythms in the Reproductive Activity of Infusoria, *Journ. Exp. Zool.,* 11: 339–359, November 20, 1911.—(17) *Id.,* The Temperature Coefficient of the Rate of Reproduction of *Paramecium aurelia, Amer. Journ. Physiol.,* 29: 147–155, December 1, 1911.—(18) *Woodruff, L. L.,* Evidence on the Adaptation of *Paramecium* to Different Environments, *Biol. Bul.,* 22: 60–65, December, 1911.—(19) *Id.,* Observations on the Origin and Sequence of the Protozoan Fauna of Hay Infusions, *Journ. Exp. Zool.,* 12: 205–264, February 10, 1912.—(20) *Fine, M. S.,* Chemical Properties of Hay Infusions with Special Reference to the Titratable Acidity and its Relation to the Protozoan Sequence, *Journ. Exp. Zool.,* 12: 2, February 10, 1912.—(21) *Woodruff, L. L.,* The Sequence of Protozoan Fauna in Hay Infusions, *Proc. Soc. Exp. Biol. and Med.,* 9: 65–66, February 21, 1912.—(22) *Woodruff, L. L.,* A Five-year Pedigreed Race of *Paramecium* without Conjugation, *Proc. Soc. Exp. Biol. and Med.,* 9: 121–123, May 15, 1912.—(23) *Baitsell, G. A.,* Experiments on the Reproduction of the Hypotrichous Infusoria. I. Conjugation between Closely Related Individuals of *Stylonychia pustulata, Journ. Exp. Zool.,* 13: 47–75, July 5, 1912.